



## Detailed assessment of microhabitat suitability for *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires, Argentina

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### Abstract

Little information is available on the ecology of *Aedes aegypti* Linnaeus at the southern extreme of its distribution (Buenos Aires, Argentina), particularly on microhabitat suitability. The aim of our study was to identify at a detailed scale, microhabitat factors that correlate with the presence of preimaginal stages of the mosquito. In March 2001, we performed a spatial census of all containers located in a 1 ha patch within a cemetery in Buenos Aires City. On a reference map (1:700) we plotted the position of graves and surrounding corridors, the location of containers, the shade projected by each plant between 10:00 and 16:00 h and vegetation cover. We classified vegetation by height, substrate by composition and shadow by level of exposure to sunlight. We performed univariate and multivariate logistic regression analyses with nine constructed independent variables, some of them at scales of 0.5, 1, 2, 3, and 10 m. Of 850 receptacles examined, 101 contained preimaginal stages of *Ae. aegypti*. Level of exposure to sunlight, type of substratum, vegetation height and distance of containers to vegetation were significantly associated with the presence of breeding sites at the studied scales. Final multivariate models were significant at scales of 2 m ( $\chi^2_{(3)} = 25.693$ ,  $p < 0.001$ ) and 3 m ( $\chi^2_{(3)} = 26.440$ ,  $p < 0.001$ ), and 65.9 and 66.8% of our data were correctly classified, respectively, for each scale. Our results suggest that sites less exposed to sunlight, with taller and closer vegetation, and in shaded and vegetated neighbourhoods were the most favourable microhabitats for *Ae. aegypti* breeding.

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### 1. Introduction

The importance of understanding vector ecology has been recognized at least since the early 1900's (Mills and Childs, 1998). Despite *Aedes aegypti* (Diptera:

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Culicidae), the vector of yellow fever and dengue, being one of the most exhaustively studied mosquitoes (Fay, 1964; Tinker, 1964; Service, 1992), its ecology remains largely misunderstood (Rodhain, 1996). Furthermore, little information is available at the southern extreme of its distribution in the American Continent (Vezzani et al., 2001), in particular at a small-scale habitat. Because *Ae. aegypti* populations from different geographical areas may differ in behaviour (Forattini, 1965), regional-specific studies on population ecology are key factors in guiding local control actions against this vector.

The environment for *Ae. aegypti* proliferation must include water-filled containers for immatures (Christophers, 1960), nectar and blood as energy source for adults (Clements, 1992; Scott et al., 1997), blood, preferentially human, for egg development (Scott et al., 1993a), and shady habitats for resting and oviposition (Horsfall, 1955; Muir et al., 1992; Scott et al., 1993b; PAHO, 1994). These requirements are fulfilled by some of the cemeteries in Buenos Aires City, thereby acting as important foci for vector proliferation (Vezzani et al., 2001). A similar situation has been documented in cemeteries in Caracas (Barrera-R et al., 1979), Manila (Schultz, 1989), Florida (O'Meara et al., 1992), and La Plata (García et al., 2002).

Microhabitat characteristics may affect the suitability of containers as breeding sites for *Ae. aegypti* (Vezzani and Schweigmann, 2002). Two of the most frequently mentioned factors influencing microhabitat quality are shade (Barrera-R et al., 1979; O'Meara et al., 1992; Tun-Lin et al., 1995; Muir and Kay, 1998) and vegetation (Focks et al., 1981; Schultz, 1989; Trpis et al., 1995; Martinez-Ibarra et al., 1997). In Buenos Aires City, we have previously found a gradient of habitat quality for *Ae. aegypti* among cemeteries, which correlated with vegetation cover (Vezzani et al., 2001).

Habitat models using presence-absence data and logistic regression are useful in formalizing the relationship between environmental conditions and habitat requirements of species, and in quantifying the amount of potential habitat (Morrison et al., 1992; Boyce and McDonald, 1999). They have been widely applied for a variety of purposes and species (e.g. Mladenoff et al., 1995; Bradbury et al., 2000; Naves et al., 2003).

In this article we performed a detailed small-scale study on habitat suitability for the presence of breeding sites of *Ae. aegypti* within one of its principal large-

scale habitats, the cemetery. The aim of our study was to identify microhabitat factors, such as shade status, vegetation height, and container availability that correlate with the presence of preimaginal stages of *Ae. aegypti*.

## 2. Materials and methods

### 2.1. Study area

Buenos Aires City (34°35'S 58°29'W) has a marked seasonal climate, with atmospheric conditions favourable for *Ae. aegypti* proliferation between September and April (de Garín et al., 2000). Chacarita, the largest cemetery of the city (72 ha), is administered by municipal authorities. It is located near the geographic centre of the city, in a crowded neighbourhood that contains a number of warehouses and important commercial activity. The study area was a patch of approximately 1 ha (48 m × 198 m) located inside the cemetery. It is characterized by graves (0.9 × 2 m each) separated by concrete paths, high availability of receptacles (approximately 1000/ha), and an average vegetation cover of 20%. We selected this study area because it showed the highest infestation rates of *Ae. aegypti* at the previous 1998–2000 period.

### 2.2. Methodology

The entire field work was conducted in March 2001. In this month the abundance of *Ae. aegypti* reaches a maximum in Buenos Aires City (Schweigmann et al., 2002). During the study period, the mean temperature was 25.6 °C (minimum: 21.5 °C, maximum: 29.7 °C) and precipitation was 100 mm (National Meteorological Service).

At the beginning of the survey, we constructed a reference map (1:700) with the position of all graves and surrounding corridors within the study patch. Graves were classified according to the type of substratum covering their surface (bare ground, short grass, long grass). The shade projected by each plant and its vegetation cover was plotted on the map. We only considered the shade projected between 10:00 and 16:00 h, because almost all the surface of the study patch was unexposed to sunshine during the rest of the day. Plants were categorized based on height as follows: less than 1 m, from 1 to less than 3 m, more than 3 m.

The locations of all containers over the graves or in surrounding paths were plotted on the map. Each container was examined by filtering the water with a fine mesh strainer, and the preimaginal mosquitoes collected were fixed in 70% ethanol. Receptacles positive for mosquitoes were distinguished on the map. Immature *Ae. aegypti* were identified using the key for Argentine mosquitoes (Darsie, 1985). Receptacles were only considered positive for *Ae. aegypti* when they contained any of the last immature stages (third and fourth instar larvae, and pupae), because these receptacles are likely to support the complete development of the mosquito. The presence of other mosquito species was disregarded.

Data were digitalized in ArcView GIS 3.1 as a raster map with a mesh size of  $0.25 \times 0.25$  m. The raster map contained a total of 152,064 cells. This spatial unit was selected, because in this case a cell did not contain more than one receptacle. For the statistical analysis contrasting the occurrence of breeding sites and independent variables, we only used data points for cells that contained receptacles, i.e., we selected cells with potential absence or presence of breeding sites. Because our data comprised more cells with absence than with presence of *Ae. aegypti*, we randomly selected cells without immatures to obtain an equilibrated sample.

### 2.3. Statistical analysis

The principle of habitat models using presence–absence data and logistic regression is to contrast

used versus unused habitat units in order to determine habitat suitability with a set of explanatory variables (Tabachnick and Fidell, 1996; Hosmer and Lemeshow, 2000).

Based on our current knowledge of *Ae. aegypti* biology, at the initial stage of our analysis we focused on variables that may influence the presence of breeding sites. In a first group we included variables describing local environmental conditions, such as vegetation height and quality of shadow and substrate, within the basic spatial unit of our analysis, a cell of  $0.25 \times 0.25$  m (cell variables, Table 1).

In a second group we constructed new variables that describe properties of the local variables on larger scales than map resolution (Schadt et al., 2002; Naves et al., 2003). The basic idea behind this concept is that the mean condition in the neighbourhood  $r$  of a cell (e.g. the mean shadow quality in the neighbourhood) may influence the habitat suitability, in addition to the local grid-scale property. Briefly, we calculated these variables using a circular moving window algorithm, assigning the mean value of the variable within the circle of radius  $r$  to the local cell. By moving the circle over the entire grid we obtained the values of the indices for each cell of the grid. Because we did not know a priori at which neighbourhood scale the variable might influence habitat selection, we calculated the neighbourhood variables for different radius  $r = 0.5, 1, 2, 3,$  and  $10$  m (neighbourhood variables, Table 1). In addition, we constructed two variables that give the distance to the nearest vegetation, including and excluding short

Table 1  
Description of the nine variables used in our analyses

Variable type	Code	Detail
Cell variables	PRES/ABS	Dependent variable, giving the presence–absence of the immature stages of <i>Ae. aegypti</i> (presence = 1, absence = 0)
	QS	Quality of shadow (direct sunlight = 1, partial shadow = 2, permanent shadow = 3)
	SUS	Quality of substrate (concrete = 1, bare ground = 2, short grass = 3, tall grass = 4)
	MVH	Maximum vegetation height (without vegetation = 1, short grass = 2, tall grass = 3, up to 1 m = 4, up to 3 m = 5, more than 3 m = 6).
Neighbourhood variables	MQS 0.5–10	Mean of shadow quality at spatial scales 0.5, 1, 2, 3, and 10 m.
	MSUS 0.5–10	Mean quality of substrate at spatial scales 0.5, 1, 2, 3, and 10 m.
	MMVH 0.5–10	Mean of maximum vegetation height at spatial scales 0.5, 1, 2, 3, and 10 m.
	MAR 0.5–10	Mean availability of receptacles at spatial scales 0.5, 1, 2, 3, and 10 m.
Distance variables	DVESG	Distance to nearest vegetation excluding short grass.
	DVISG	Distance to nearest vegetation including short grass.

Values of cell variables are given for each category.

grass. Short grass is not tall enough to give shade to the receptacles (distance variables, Table 1). In total, we obtained a total of nine variables and four of these, the neighbourhood variables, at five different scales (Table 1).

Spatial autocorrelation in the dependent variable can lead to pseudo replication problems because data are not independent and hence the increased power produces overfitted models with little biological interpretation (Lennon, 1999). To test for spatial independence we calculated a coefficient  $c(r)$ , which describes the autocorrelation of a variable  $x$  with lag  $r$ , inside the area that comprises cells with observations and non-observations. To reduce problems with severe spatial correlation between cells, we determined the spatial lag at which locations were not strongly correlated [i.e.,  $c(r) < 0.7$ ] and only used sufficiently separated cells (Schadt et al., 2002; Naves et al., 2003).

At the beginning of data analysis ‘sensu stricto’, we used a descriptive analysis for each independent variable to test the differences between cells with and without *Ae. aegypti*. For this purpose we used a Mann-Whitney test (Siegel and Castellan, 1995). With those variables that showed significant differences we additionally performed a univariate logistic regression to assess how well each individual variable predicted the presence or absence of *Ae. aegypti*.

We tested for correlation between the remaining variables that had shown significant differences in the logistic models (i.e.,  $p < 0.05$ ). We avoided strong multicollinearity between the explanatory variables by choosing the variable that correlated most strongly with the dependent variable of the univariate logistic regression. We considered two independent variables to be strongly correlated when the Pearson correlation coefficient was  $r_p > 0.5$ , as determined by the correlation matrix of the predictors (Spearman-Rho, two-tailed).

Finally, we performed stepwise multivariate logistic regression analyses with the remaining independent variables. Significance of the models was assessed using the Wald statistics (Hosmer and Lemeshow, 2000).

### 3. Results

Of a total of 850 receptacles examined, 101 contained preimaginal stages of *Ae. aegypti*, yielding an

infestation rate of 11.9%. Spatial correlation coefficients  $c(r)$  of the dependent variable (presence or absence of the immature stages of the mosquito) were below 0.40 ( $p < 0.05$ ) for all spatial scales  $r$  studied (0.5–10 m), while those of the independent variables were below 0.70. Therefore, it was not necessary to eliminate data points to avoid spatial autocorrelation of the dependent and the independent variables.

From the  $n = 749$  receptacles without *Ae. aegypti*, 101 were selected randomly for further analysis to obtain an equilibrated sample. The descriptive univariate analysis for several variables showed significant differences between presence and absence of breeding sites (Table 2). For the three cell variables there were highly significant differences between the presence and absence of *Ae. aegypti*; in cells with presence the quality of shadow and the quality of substrate was higher, and the maximum vegetation height was taller than in cells lacking breeding sites. Additionally, the former were shadier (mean of shadow quality) and had taller vegetation (mean of maximum vegetation height) in all five 0.5–10 m neighbourhoods. Cells with *Ae. aegypti* had more receptacles in their 10 m neighbourhood (mean availability of receptacles). However, the mean availability of receptacles at 0.5–3 m, and the mean quality of the substrate at 0.5–10 m showed no significant differences between presence and absence of breeding sites. The variable describing the distance to nearest vegetation that included short grass showed no significant difference between presence and absence of breeding sites, while there were significantly more cells with *Ae. aegypti* closer to vegetation excluding short grass.

All variables that showed significant differences between presence and absence of *Ae. aegypti* yielded also significant univariate logistic models (Table 2), except for the mean availability of receptacles at 10 m. The classification tables showed relatively little difference in the percentage of cases correctly classified among models (min = 55.94%, max = 62.87%). In general, neighbourhood variables with smaller neighbourhoods showed stronger association with the dependent variable.

We eliminated the variable maximum vegetation height for further analysis because it was highly correlated with the quality of shadow ( $r_p = 0.732$ ). We also found strong correlations between the corresponding neighbourhood variables (mean of maximum

Table 2  
Univariate statistics for the independent variables

Variable	Descriptive univariate analysis						Logistic univariate models				
	Without <i>Ae. aegypti</i> breeding sites		With <i>Ae. aegypti</i> breeding sites			Significance	$\beta \pm$ S.E.	Wald	df	Significance	% observed correctly classified
	Median	LQ-UQ	Median	LQ-UQ	U						
QS	2.00	1.00–3.00	3.00	2.00–3.00	3661	***	0.66 ± 0.18	14.1	1	***	60.9
SUS	3.00	2.00–3.00	3.00	3.00–4.00	4125	**	0.51 ± 0.20	7.0	1	**	55.9
MVH	2.00	2.00–3.00	3.00	2.00–4.00	3748	***	0.31 ± 0.10	9.2	1	**	60.9
MQS05	0.68	0.00–1.23	1.19	0.28–1.82	3790	***	0.62 ± 0.20	10.0	1	**	58.9
MQS1	0.61	0.00–1.22	1.17	0.32–1.53	3757	***	0.70 ± 0.22	10.4	1	**	60.4
MQS2	0.56	0.00–1.05	0.97	0.40–1.33	3769	***	0.80 ± 0.25	10.4	1	**	62.9
MQS3	0.52	0.04–0.87	0.83	0.35–1.19	3901	**	0.80 ± 0.26	9.2	1	**	60.4
MQS10	0.31	0.13–0.76	0.52	0.21–1.01	4223	*	0.69 ± 0.32	4.7	1	*	57.9
MSUS05	1.46	0.90–1.77	1.58	0.90–1.90	4604		–	–	–	–	–
MSUS1	1.22	0.73–1.54	1.31	0.82–1.61	4741		–	–	–	–	–
MSUS2	1.28	0.62–1.51	1.31	0.87–1.52	4908		–	–	–	–	–
MSUS3	1.16	0.59–1.35	1.21	0.87–1.38	4822		–	–	–	–	–
MSUS10	0.73	0.50–0.93	0.79	0.57–1.01	4579		–	–	–	–	–
MMVH05	0.85	0.00–1.50	1.33	0.28–2.09	3956	**	0.28 ± 0.10	7.2	1	**	59.4
MMVH1	1.66	1.00–2.39	2.14	1.46–2.82	3990	**	0.22 ± 0.09	5.9	1	*	60.9
MMVH2	0.67	0.05–1.20	0.96	0.59–1.58	3967	**	0.31 ± 0.13	6.3	1	*	56.4
MMVH3	0.63	0.14–0.93	0.85	0.48–1.34	4035	**	0.34 ± 0.14	5.8	1	*	57.4
MMVH10	0.38	0.17–0.88	0.68	0.28–1.31	4246	*	0.47 ± 0.24	4.1	1	*	57.4
MAR05	0.14	0.08–0.15	0.14	0.08–0.15	5015		–	–	–	–	–
MAR1	0.04	0.03–0.06	0.04	0.03–0.07	4800		–	–	–	–	–
MAR2	0.02	0.01–0.03	0.02	0.01–0.03	4769		–	–	–	–	–
MAR3	0.01	0.00–0.02	0.01	0.01–0.02	4664		–	–	–	–	–
MAR10	0.01	0.00–0.01	0.01	0.00–0.01	4280	*	59.3 ± 34.5	2.9	1	–	–
DVESG	1.00	0.00–2.62	0.00	0.00–1.50	4223	*	-0.25 ± 0.08	9.8	1	**	56.4
DVISG	0.00	0.00–0.20	0.00	0.00–0.00	4567		–	–	–	–	–

Descriptive univariate analysis: the medians, lower quartiles (LQ) and upper quartiles (UQ) of the distribution are given for each variable. \*\*\* Indicates differences of the Mann-Whitney test (U) at a significance level of  $p < 0.001$ . \*\* At a significance level of  $p < 0.01$ . \* At a significance level of  $p < 0.05$ . Logistic univariate models: results of the univariate logistic regressions, cut level = 0.5. \*\*\* Indicates that the variable is entered at a significance level of  $p < 0.001$ . \*\* At a significance level of  $p < 0.01$ . \* At a significance level of  $p < 0.05$  following the Wald statistics.

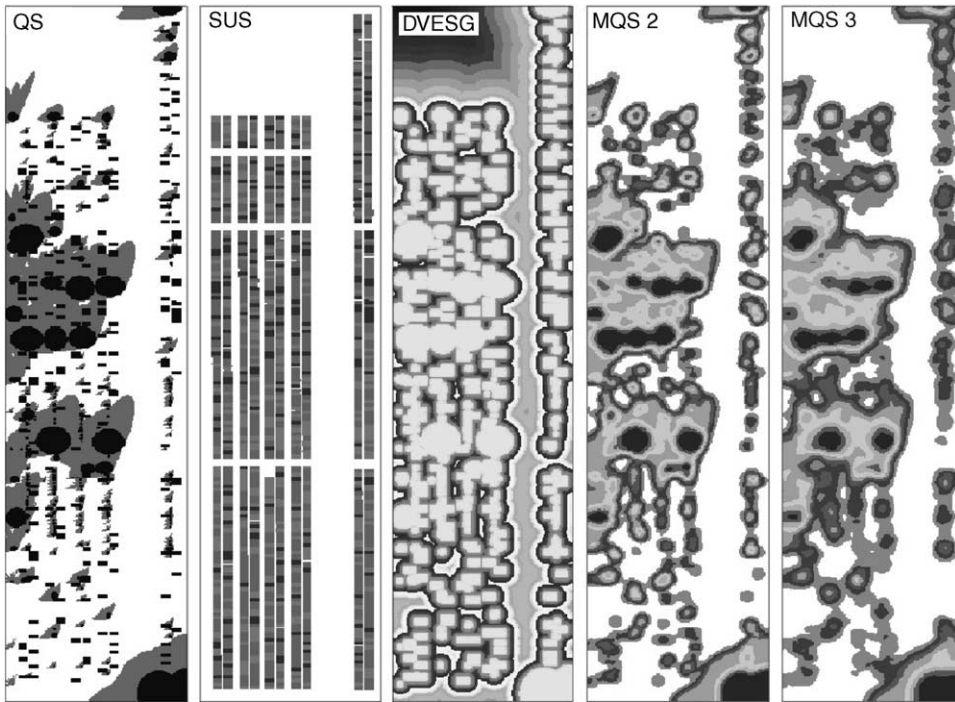


Fig. 1. The study patch plotted (1:700) for two cell variables (QS and SUS), one distance variable (DVESG) and one neighbourhood variable at scales of 2 and 3 m (MQS2 and MQS3, respectively).

vegetation height and mean of shadow quality) at the spatial scales 0.5, 1, 2, 3, and 10 m ( $r_p = 0.844, 0.895, 0.860, 0.870$  and  $0.975$ , respectively). We retained the latter variable because it was more strongly associated with the dependent variable than the former one.

In summary, we identified four independent variables: two cell variables (quality of shadow and quality of substrate), one neighbourhood variable at five different spatial scales (mean of shadow quality), and one variable describing distance to the nearest vegetation excluding short grass. With these variables (Fig. 1) we constructed five multivariate regression models, one for each spatial scale of the neighbourhood variable. At spatial scales of 2 and 3 m we obtained significant models with more than one independent variable (scale 2 m:  $\chi^2_{(3)} = 25.693$ ,  $p < 0.001$ ; scale 3 m:  $\chi^2_{(3)} = 26.440$ ,  $p < 0.001$ ), being 65.9 and 66.8% of data correctly classified for each scale, respectively. The variables that entered significantly were quality of substrate, distance to the nearest vegetation excluding short grass and mean of shadow quality (2 or 3 m) (Table 3), the quality of shade QS did not enter in the model. For the other

Table 3

Results of significant multivariate logistic regression analyses, cut level = 0.5

Scale	Variable	$\beta \pm \text{S.E.}$	Wald	df	$p$
2 m	SUS	$0.4503 \pm 0.2108$	4.5650	1	0.0326
	DVESG	$-0.1857 \pm 0.0860$	4.6674	1	0.0307
	MQS2	$0.7530 \pm 0.2574$	8.5578	1	0.0034
3 m	SUS	$0.4716 \pm 0.2128$	4.9094	1	0.0267
	DVESG	$-0.2049 \pm 0.0874$	5.4952	1	0.0191
	MQS3	$0.8408 \pm 0.2780$	9.1485	1	0.0025

spatial scales (0.5, 1, and 10 m) the stepwise procedure stopped at step one, yielding univariate models with variable QS that correctly classified 60.9% of the data (Table 2).

#### 4. Discussion

The abundance of *Ae. aegypti* recorded in the study area is within the range documented previously for Buenos Aires; it is half the maximum infestation level

detected within a cemetery (25% of the containers with immatures of the mosquito; Vezzani et al., 2001), and twice the mean value registered in houses of this city (6% of the containers with immatures of the mosquito; Cardinal et al., 2000).

We found that six of the nine studied variables (QS, SUS, MVH, MQS, MMVH, DVESG) were indeed significantly associated with the occurrence of breeding sites at the scale studied, and no associations were found for the other three variables (MSUS, MAR, DVISG). These significant associations represent the effects of the shade provided by the vegetation and/or the presence of vegetation itself on the suitability of the microhabitat. Our results suggest that the most favourable microhabitats for *Ae. aegypti* breeding were those with lesser sun exposure, with taller and closer vegetation, and with shadier and more vegetated neighbourhoods. In Buenos Aires, the importance of vegetation in the habitat quality for this insect was previously reported at two less-detailed spatial scales and for larger areas: among cemeteries (Vezzani et al., 2001) and between two huge urban areas of the city (Schweigmann et al., 2002).

Our final logistic model contained three variables: the quality of substrate (SUS) with positive sign, the distance to nearest vegetation excluding short grass (DVESG) with negative sign, and the average shadow quality at spatial scales 2 and 3 m (MQS2 and MQS3) with positive sign. The two final models explained 66.8 and 65.8% of our data correctly, only 5–6% more than the univariate model with shadow quality QS as predictive variable. This result suggests that shadow is the main factor determining breeding site quality in this particular environment. The neighbourhood variables at smaller scales (0.5–3 m) were more strongly associated with the occurrence of *Ae. aegypti* immatures than those at the larger (10 m) scale. Thus, vegetation and shade in the immediate proximity of a given site have a stronger influence on microhabitat suitability than at larger distances.

Of equal interest are the identifications of variables that did not show significant association with *Ae. aegypti* breeding sites. For example, the availability of receptacles in the neighbourhood (MAR) did not differ significantly between sites with and without *Ae. aegypti*. However, according to Moore et al. (1978) the abundance of *Ae. aegypti* is proportional to the availability of potential breeding sites, and Tinker (1964)

found a positive association between the infestation rates and the density of containers. Our negative result may be due to the high density of containers, a common fact for cemeteries (Vezzani et al., 2001). An additional factor to keep in mind for future studies is the high habitat connectivity within a cemetery that enhances the access of gravid females to the containers, in contrast to the fragmented urban landscape where the dispersal among premises and blocks is more complicated. Short grass in the neighbourhood is another factor unsuitable for *Ae. aegypti* development. This result was obtained by comparing the variables DVISG and DVESG, which describe the distance to the nearest vegetation including and excluding short grass, respectively.

The shade may buffer high temperatures and low humidities, which are unfavourable for the survival of adults and larvae (Fay, 1964; Tun-Lin et al., 2000), thus improving habitat quality. In New Orleans, Focks et al. (1981), found that unshaded locations are somewhat less likely to breed mosquitoes, possibly because of high temperatures. A higher frequency of positive containers in shaded habitats has been documented worldwide. Some examples are Australia (Tun-Lin et al., 1995; Muir and Kay, 1998), United States (Focks et al., 1981), Mexico (Espinoza Gómez et al., 2001), Thailand (Kittayapong and Strickman, 1993), and Venezuela (Barrera-R et al., 1979).

Shaded containers are generally assumed to be preferred by *Ae. aegypti* for oviposition (Christophers, 1960; Nelson, 1986; PAHO, 1994). Furthermore, Fay and Eliason (1966) and Evans and Bevier (1969) recommended not to expose ovitraps to sunlight. However, more recent studies (Chadee, 1992; Almirón et al., 1999) conclude that exposure to direct sunlight is irrelevant for oviposition. In our study, we are unable to evaluate if shadow influences the selection of oviposition sites or favours the survival of eggs and larvae after oviposition.

The individual effect of shadow and vegetation is hard to discriminate in an environment where all shaded sites result from vegetation. In addition to shade, vegetation may have other favourable effects on microhabitat suitability by providing containers with detritus (including leaves) as food for larvae (Clements, 1992; Walker et al., 1997), and nectar and honeydew as carbohydrate sources for adults (Trpis et al., 1995; Martínez-Ibarra et al., 1997; Briegel et al., 2001). Considering that in cemeteries the visitors bring in numerous fresh-

cut flowers, we assume that detritus and carbohydrate are not limiting factors for *Ae. aegypti* in the study area, and that the main importance of the vegetation studied (trees, shrubs and grass) is to provide shadow to microenvironments.

In this particular environment, measuring sunlight levels over the whole landscape appears to be the best cost–benefit relationship to compare suitability among microhabitats. Further studies are needed to explore the extension of our results to a typical urban environment, like a neighbourhood.

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